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The histopathology of acute pasteurellosis in mature chickens.

Keith Ray Rhoades
Iowa State University

THE HISTOPATHOLOGY OF ACUTE PASTEURELLOSIS IN MATURE CHICKENS

by

Keith Ray Rhoades

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INTRODUCTION

In epornitics of fowl cholera (avian pasteurellosis) the acute form is commonly observed. Often birds affected with this form of the disease are found dead with no premonitory evidence of disease, and with few, if any, gross lesions.

Relatively little information is available on the histopathology of the acute form of the disease, and consequently the pathologic changes principally responsible for death remain obscure.

The objectives of this experiment were to describe the histopathologic changes of acute fowl cholera in adult chickens, and to gain insight on the changes which lead to death by characterization of the tissue response observed.

REVIEW OF LITERATURE

Fowl cholera is a contagious bacterial disease caused by Pasteurella multocida. It has been reported in all species of domestic poultry, game birds, waterfowl, seagulls, canaries, small feral birds, and birds of prey (1). All species of birds are probably susceptible (1). Its distribution is widespread (1, 2).

This disease has been a problem for poultry raisers and a subject of study for research workers for a long time. Chabert, according to Salmon (3), studied it in 1782 and erroneously regarded it as a form of anthrax. The disease was a problem in France as early as 1825 (3). According to Kaupp (4), the first study of avian pasteurellosis in the United States was by Salmon in 1880.

The effect of the infection on the bird varies from death with little or no clinical signs of disease to localized suppurative alterations of long duration (2).

The gross lesions of avian pasteurellosis vary also, with the type of lesion observed being related to the course of the disease. Often lesions are not found in birds which die with no premonitory signs (2, 5, 6).

The gross lesions most often observed in postmortem examination of birds which have died from the acute form of the disease are: petechial hemorrhages in the lungs and intestinal

mucosa (2, 5, 6), hemorrhages in serous membranes (2, 5, 6), petechial hemorrhages on the heart (2, 4, 6, 7), fibrinous exudate in the pericardial sac (5, 6, 7), parenchymatous hepatitis (2, 4, 5) often with focal areas of hepatic necrosis (2, 5, 6, 7), hemorrhagic enteritis (4, 6, 7) especially of the duodenum (5), ovarian follicle rupture in laying hens (4), pulmonary hyperemia (6, 7), and cyanosis of the head and wattles (4).

The lesions most often associated with the chronic form of the disease are: emaciation (6), caseous necrosis in the lungs, liver and joints (6, 7), fibrinous pleuritis and pericarditis (7), catarrhal inflammation in the nasal passages and trachea (2, 5), localized infection of the wattles (2, 7), caseous inflammation in the internal ear or in the bones at the base of the brain (2, 5), suppurative inflammation of the oviduct (8), and osteomyelitis (8).

Relatively little information is available on the lesions of avian pasteurellosis as determined by microscopic examination. Salmon, in 1889, (3) described the microscopic observation of subserous hemorrhages in sections of proventriculus. Kaupp (4) described the histopathology of fowl cholera as follows:

> There is a slight congestion of the heart, with a tendency for the muscle fibers to lose their cross-striation. The liver shows both active and passive congestion. The kidneys show cloudy swelling and areas of focal necrosis are present. Some cells are in a state of pyknosis; in some areas, the cells leave their base, passing to the center and obliterating the

lumen of the tubule; in other areas the nuclei and cells are in a state of disintegration. Active and passive congestion are present.

In 1935 Barboni and Tolomei (9) studied the pathologic changes in the livers and hearts of 12 chickens affected with acute fowl cholera and described the following lesions:

> Pericardial fluid was always present in excess, the heart muscle was swollen, intensely congested and sometimes contained small haemorrhages. In two cases, necrotic foci were present in the epicardium together with an accumulation of a gelatinous substance. All the livers were congested and oedematous; fatty degeneration was present and in some cases necrotic foci in various stages of gelatinous degeneration were also seen. In these cases, the gelatinous material often invaded the neighbouring parenchyma. Small haemorrhages were also sometimes present on the surface of the liver. The portal vessels frequently contained thrombi formed of closely packed erythrocytes which also showed various stages of gelatinous degeneration; a perivascular inflammatory infiltration was also present. The changes in the liver may, in short, be described as a degenerative or necrotic parenchymatous hepatitis.

Barboni and Arangio (10) examined material collected from 18 cases of acute fowl cholera and described the lesions which occurred in the brain, kidneys, intestines, spleen and pancreas as follows:

> The dura mater always appeared normal, but the pia mater and the brain showed inflammatory changes consisting of vascular congestion and the extravasation of fluid, containing neutrophile and mononuclear cells, into the surrounding tissues. Inclusion bodies were never observed. The kidneys were swollen, soft and greyish-red in colour with small pin-point haemorrhages under the capsule, and microscopic examination revealed the presence of an acute glomerulonephritis. All the blood vessels were congested, some contained emboli and there were scattered haemorrhages in Bowman's capsule and the glomeruli and degeneration of the vessel walls. In addition, the tubules were dilated, their epithelium swollen and albumin casts were

> often found in them which, together with degeneration of the interstitial cells, were the chief changes seen in the kidneys. The spleen was always slightly enlarged, reddish-brown in colour and showed changes typical of a splenic infection. The whole of the small intestine was affected by an acute catarrhal enteritis, the lesions being most pronounced in the duodenum and usually localized to the mucous membrane or sub-mucous layer. Finally, the pancreas was oedemic and congested and degenerative changes which never involved the islets of Langerhans were constantly present.

The method by which Pasteurella multocida produces lesions in affected animals has not been determined. Hutyra, Marek, and Manninger (6) attribute the pathological processes to the action of an unknown toxic substance.

Movsesyan (11, 12) has reported that there is a relationship between lesions in the central nervous system and lesions in other organs of cattle infected with pasteurellosis. He believes that the central nervous system plays a role of regulator in the pathogenesis of the disease.

Stamatin et al., (13) studied experimental pasteurellosis in sheep and reported that death resulted from hypoxia which they attributed to blockage of pulmonary vessels by leucocytes.

METHOD OF PROCEDURE

Twelve mature disease free New Hampshire Red chickens, 6 males and 6 females, were used in the experiment. Three chickens of each sex were exposed by nasal cleft instillation of 0.1 ml. of a suspension of Pasteurella multocida[1] of avian origin (14). The bacteria used as inoculum were grown on Dextrose Starch Agar plates[2] and suspended in Tryptose Broth.[2] The concentration of bacteria in the suspension was adjusted to a density that allowed 75% light transmission at 600 millimicron wavelength in a colorimeter.[3] This concentration contains approximately 5.4×10^8 organisms per ml. as determined by the average plate count of 5 similar suspensions. The 6 unexposed chickens were used as normal controls for evaluating the changes produced by the infection.

Signs of disease observed during the course of the infection, and gross lesions observed immediately after death were recorded. The control birds were killed by electrocution and immediately examined for lesions.

The liver and breast muscle of each of the exposed and each of the control birds were examined for bacteria. The

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[1]Pasteurella multocida (X-73) secured from K. L. Heddleston, National Animal Disease Laboratory, Ames, Iowa.

[2]Difco Laboratories, Detroit 1, Michigan.

[3]Spectronic 20 Colorimeter, Bausch and Lomb Incorporated, Rochester 2, New York.

surface of the tissue to be cultured was seared with a heated spatula and a sterile cotton swab was introduced through the seared surface into the underlying tissue. The swabs were then used to streak dextrose starch agar plates. The plates were incubated at 37°C and were examined for evidence of bacterial growth.

Tissues from both the exposed and control birds were processed for microscopic examination. Thirty-eight different blocks of tissue were prepared from each bird. Central nervous system tissues were fixed in 25% formalin in tap water, all other tissues were fixed in 10% formalin. Osseous tissues were decalcified in 30% formic acid. Paraplast[1] was used as an infiltrating and embedding material. Sections were cut 6 microns thick using a rotary microtome.[2] The stains and staining procedures used are described in the manual of histologic and special staining techniques of the Armed Forces Institute of Pathology (15).

In the following list are the tissues which were included in the study and the stains used for each. The stains are indicated by the following symbols; (a) hematoxylin and eosin, (b) Giemsa, (c) Gomori's trichrome, (d) mucicarmine, (e) luxol-fast-blue periodic-acid-Schiff hematoxylin, and (f) MacCallum-Goodpasture stain for bacteria.

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[1]Aloe Scientific, 3501 Raleigh Ave., So., Minneapolis 16, Minn.

[2]Model 820, American Optical Company, Buffalo 15, New York.

Nervous system	
brain	(a,e,f)
spinal cord	(a,e)
Digestive system	
esophagus	(a,d)
crop	(a,d)
proventriculus	(a,b,d)
gizzard	(a,c)
small intestine	(a,b,c,d)
caeca	(a,b,d)
colon	(a,b,d)
liver	(a,b,c)
pancreas	(a,b,c)
mesentery	(a,b)
Circulatory system	
heart	(a,c)
spleen	(a,b)
Skeletal system	
humerus	(a,b)
femur	(a,b)
Respiratory system	
nasal cleft	(a,b)
trachea	(a,c,d)
lungs	(a,b,c,d,f)
air sac	(a,c)
Endocrine system	
adrenal	(a,b,c)
thyroid	(a,c)
thymus	(a,b)
Uro-genital system	
kidney	(a,b,c)
ovary/testicle	(a,b)
oviduct	(a,b)
Integumentary system	
conjunctiva	(a)
wattle	(a)
sternal bursa	(a,b)
Muscular system	
skeletal muscle	(a,c)

All sections were examined microscopically and the lesions were recorded.

RESULTS

Signs of Disease

The first sign of disease in the exposed birds was depression. The hens first appeared depressed 18 hours after exposure, the roosters approximately 1 hour later. The degree of depression increased during the course of the disease until the birds were comatose shortly prior to death.

Diarrhea was observed in two of the roosters approximately 19 hours after exposure. The fecal material from these birds was viscid and resembled mucus.

The respiratory rates of each of four birds observed a few minutes prior to death were increased. They ranged from 50 to 92 per minute. Several normal respiratory rates for chickens are presented by various workers (16). They range from 12 to 21 per minute for males and from 20 to 37 for females.

The three exposed hens died 20, 22, and 22½ hours after exposure, the roosters died 25, 26, and 28 hours after exposure. Those birds observed at the time of death exhibited agonal convulsions and pronounced cyanosis of the comb and wattles.

Bacteriology

Examination of the dextrose starch agar plates which were streaked with tissues from the control birds did not reveal the presence of bacteria. Examination of those from the exposed

birds revealed heavy bacterial growth. All colonies appeared to be of the same type, and were fluorescent when viewed with oblique lighting.

Biochemically the bacteria isolated from the exposed birds were identical with those used as inoculum. They produced acid in dextrose, sucrose, dulcitol, and arabinose, and did not produce acid in lactose, maltose, xylose, or trehelose. Indol was produced when the bacteria were grown in tryptophane, as was a slight amount of hydrogen sulfide.

Pathology

Circulatory system

Gross examination of the blood vascular system of the infected birds revealed a generalized distention of the veins with blood. This congestion was especially evident in the veins of the abdominal viscera. The middle third of the jejuno-ileal portion of the small intestine of each of the infected males was markedly congested.

Blood vessels cut during examination of the non-infected birds bled more freely and for a longer period of time than those of the birds which died as a result of infection, even though both were examined approximately 15 minutes after death.

Microscopically there was a generalized hyperemia involving the veins, venules and capillaries of the infected birds. Although all tissues were involved, the most marked congestion

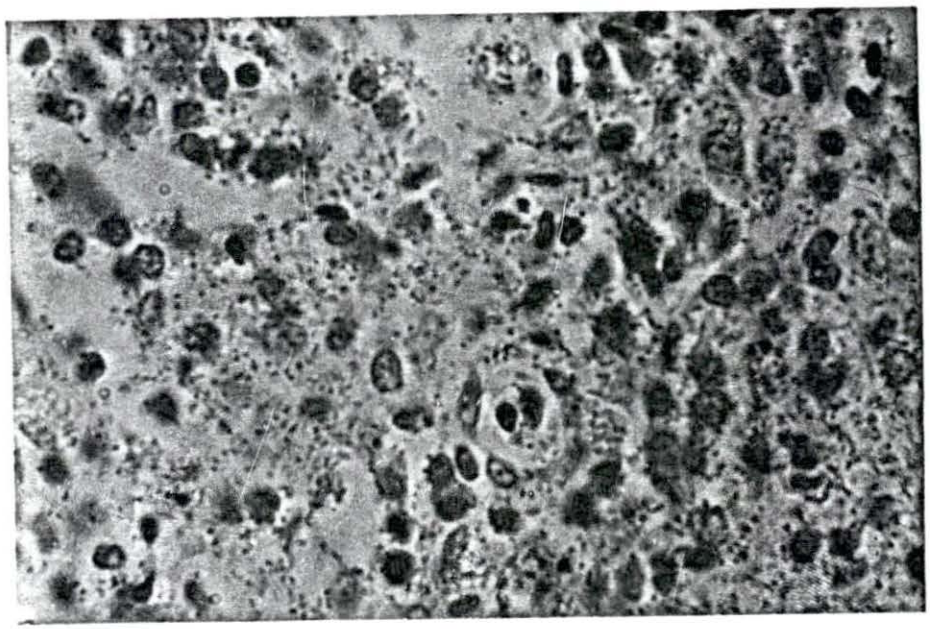
was of the mid-portion of the small intestine of the males. The normal cytologic character of the lamina propria in some areas of this portion of the intestine was obscured by the engorged small vessels. In contrast, the same areas in the intestines of the infected females were only moderately hyperemic. Marked vascular congestion was also observed in the liver and mesentery of each of the infected birds. In general the hyperemia was less pronounced in the females than in the males.

Bacteria were observed in the blood vessels of all sections from the infected birds. They were not, however, evenly distributed. Some veins contained great numbers of bacteria while other veins of approximately the same size and in the same tissue contained relatively few bacteria.

The spleens of the infected birds appeared to be of normal size, color and consistency when examined grossly. Microscopically the veins were slightly distended and the sinusoids contained more blood than did those of the control birds. There was little or no change in the number of heterophils distributed throughout the red pulp. Bacteria were very numerous in the red pulp (Figure 1). Relatively few were found in the areas of white pulp.

No cardiac or pericardial lesions were observed grossly. Microscopically the coronary veins and capillaries of the infected birds were dilated with blood and contained bacteria.

Figure 1. Spleen of a hen (no. 52) which died from acute fowl cholera. Numerous bacteria are present. Giemsa stain x 1120.



No other lesions were observed.

Gross observation of the femurs and humeri of the infected chickens did not reveal lesions. Microscopically there was a very marked depletion of heterophils in the marrow of the infected males (Figure 2), as compared to the controls (Figure 3), and a moderate depletion of these cells in the infected females. Many of the hemopoietic cells of the marrow of the infected birds had undergone degenerative changes indicated by pyknosis. The marrow of the control roosters contained many more heterophils and less fat than the marrow of the control hens. The veins and sinusoids of the marrow from the infected birds were congested with blood. Bacteria were numerous, especially in the sinusoids.

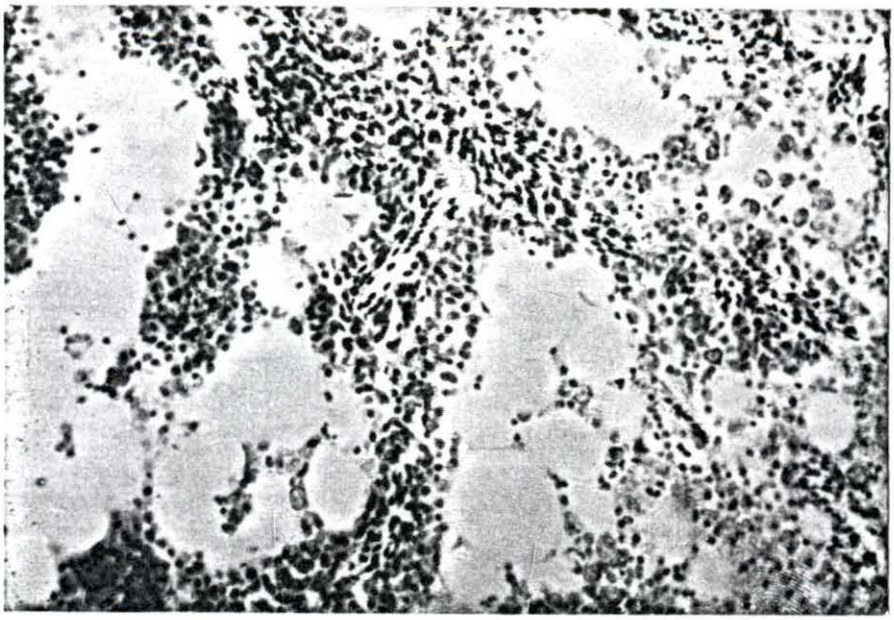
No histopathologic changes were observed in the lymphoid nodules of the infected chickens.

Digestive system

Gross examination of the digestive tracts of the infected chickens revealed generalized venous congestion and increased amounts of mucus. The lumen of the mid-portion of the small intestine of each infected male contained a small amount of blood as well as mucus. The walls of the intestine in this area were markedly congested (Figure 4). The serosal surface of the gizzard of one infected rooster contained a few small areas of hemorrhage. More abdominal fat was present in the hens than in the roosters.

Figure 2. Bone marrow from a rooster (no. 31) which died of acute fowl cholera. Very few heterophils are present. Giemsa stain x 280.

Figure 3. Bone marrow from a control rooster (no. 35). Numerous heterophils are present. Giemsa stain x 280.



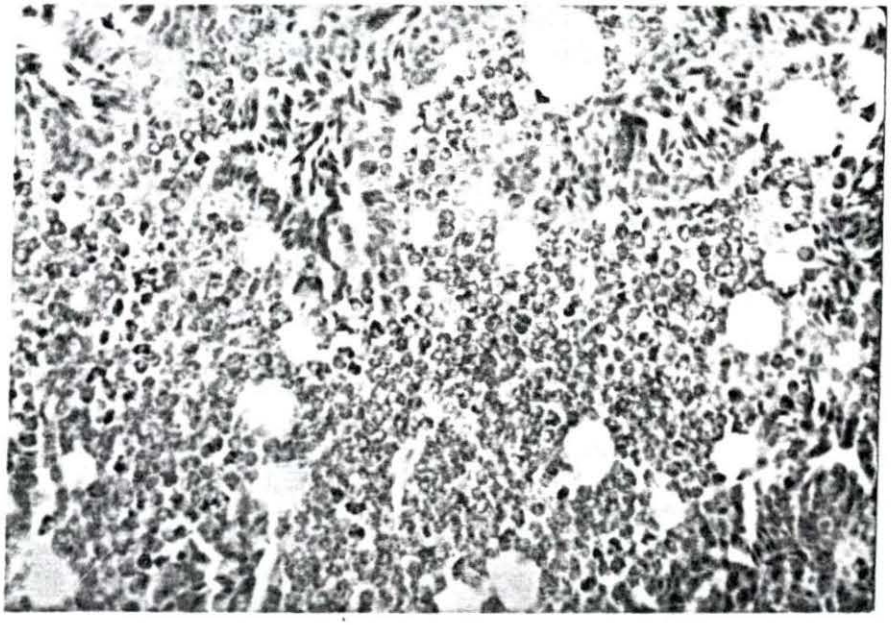
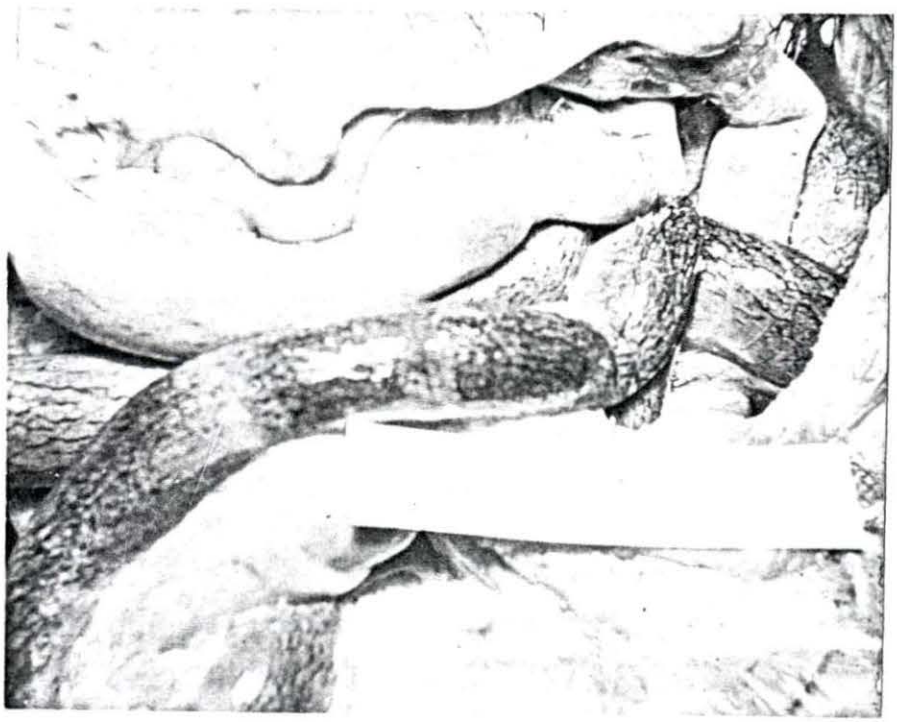


Figure 4. Marked congestion of the middle of the jejunoileal portion of the small intestine of a rooster (no. 31) which died of acute fowl cholera.



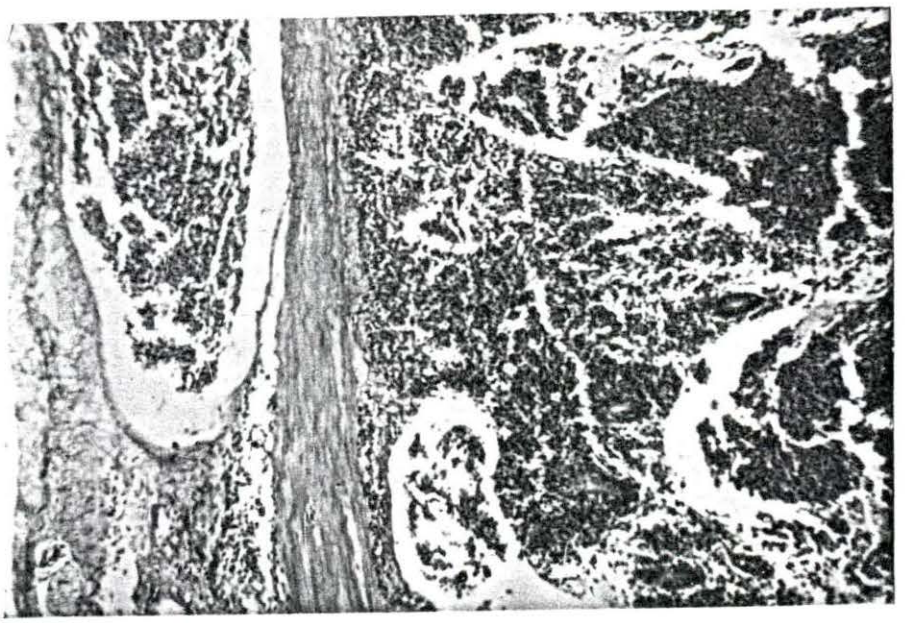
Microscopic examination revealed vascular congestion and intravascular bacteria throughout the digestive tracts of the infected birds. The vessels of the middle third of the jejuno-ileal portion of the small intestine of each infected male were engorged with blood (Figure 5). Vessels in the same area in the noninfected birds contained relatively little blood (Figure 6). Sections stained with mucicarmine did not reveal evidence of increased mucus. The walls of the intestines of the hens contained more fat than those of the roosters.

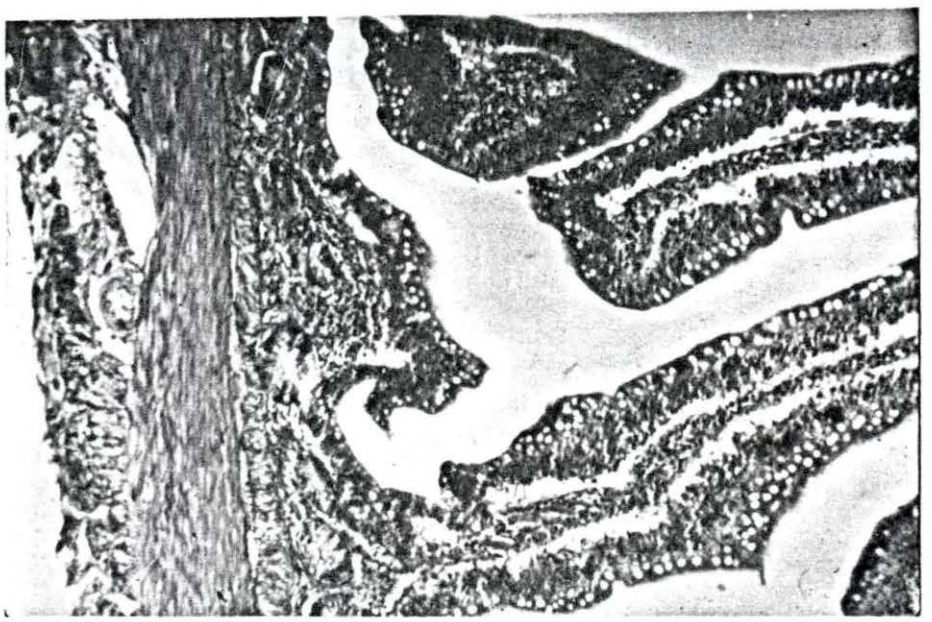
Multiple small necrotic areas 1 to 2 millimeters in diameter were observed in the livers of the infected chickens (Figure 7), but there was no apparent change in size or shape. The livers of the control and infected hens were lighter brown in color than those of the males. They were also more friable than the livers of the males.

Microscopic examination of the livers from the infected birds revealed an acute focal necrotic hepatitis characterized by multiple focal areas of coagulative necrosis and heterophilic infiltration. There was a marked concentration of heterophils in and immediately around the necrotic areas (Figures 8 and 9) as well as generalized infiltration. Very few heterophils were observed in the livers of the control birds (Figures 10 and 11). The hepatic veins were congested and the sinusoids contained more blood than did those of the control birds.

Figure 5. Marked vascular congestion of the small intestine of a rooster (no. 31) which died of acute fowl cholera. Trichrome stain. x 280.

Figure 6. Small intestine of a control rooster (no. 33). Trichrome stain. x 280.





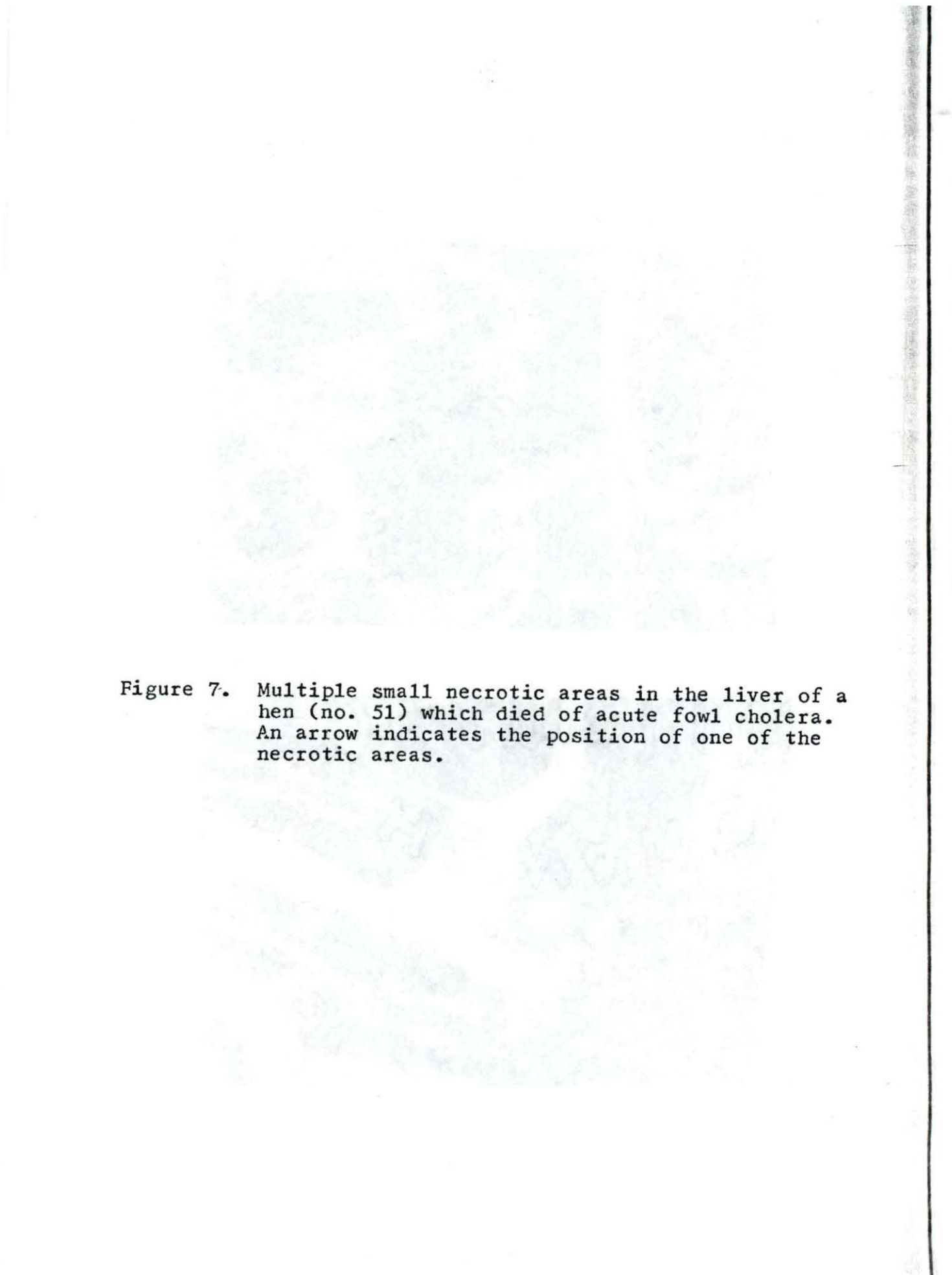


Figure 7. Multiple small necrotic areas in the liver of a hen (no. 51) which died of acute fowl cholera. An arrow indicates the position of one of the necrotic areas.

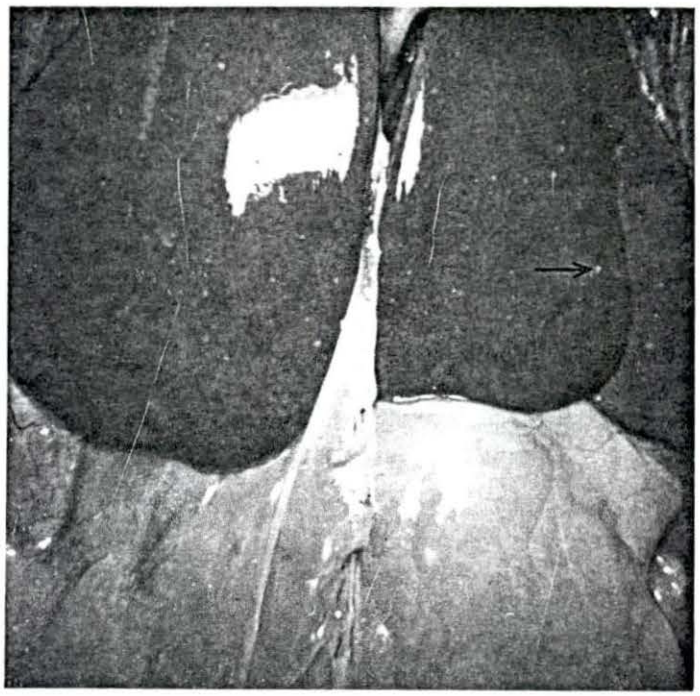
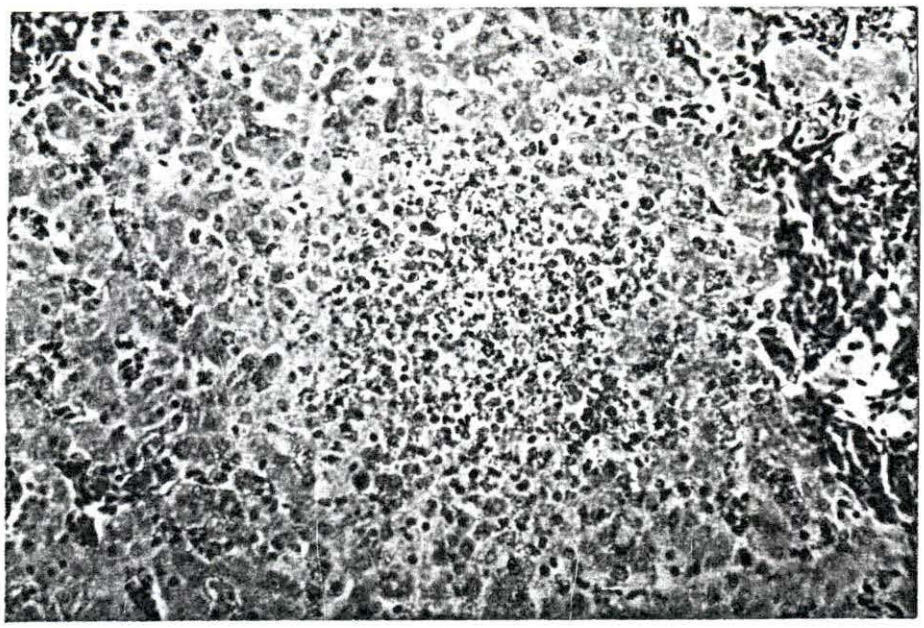


Figure 8. Coagulative necrosis accompanied by marked heterophilic infiltration in the liver of a rooster which died of acute fowl cholera. Giemsa stain. x 280.

Figure 9. Marked heterophilic infiltration and focal necrosis in the liver of a hen which died of acute fowl cholera. Giemsa stain. x 280.



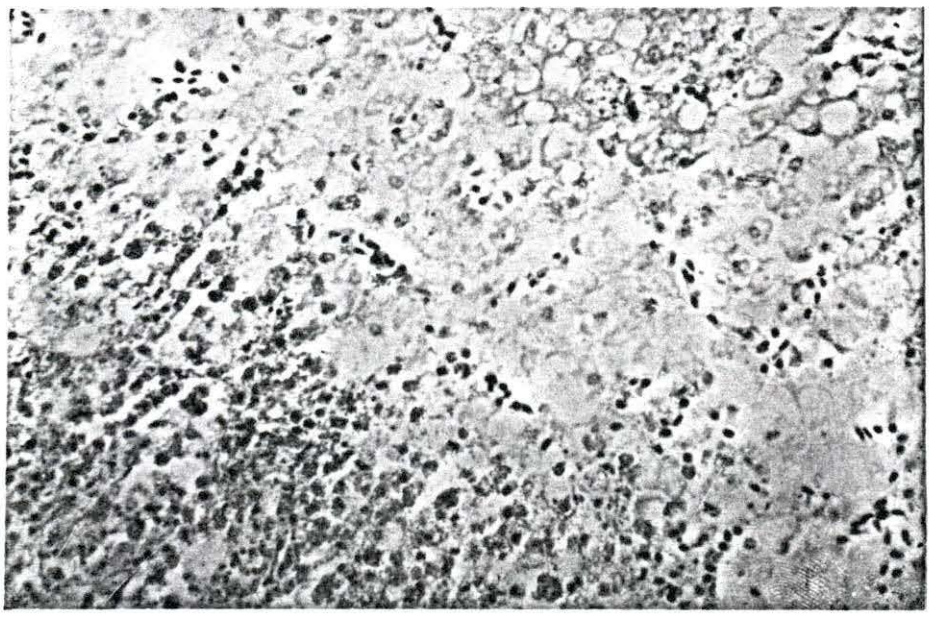
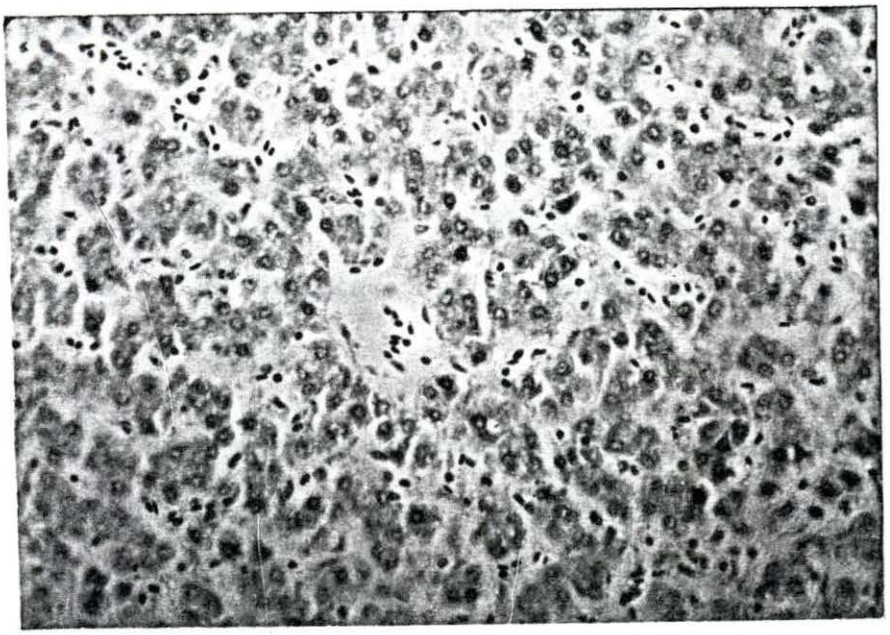
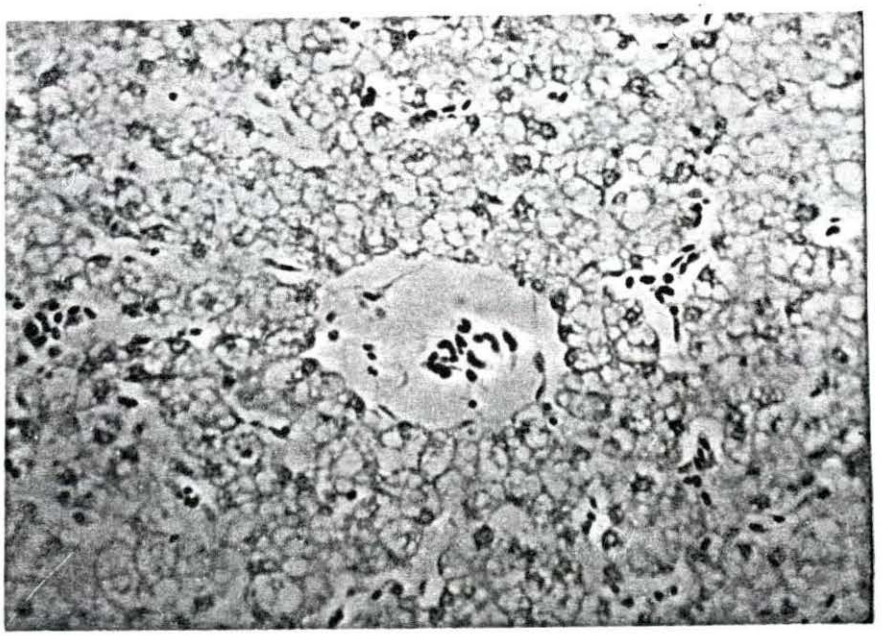


Figure 10. Liver from a control rooster (no. 33). Giemsa stain. x 280.

Figure 11. Liver from a control hen (no. 1). Giemsa stain. x 280.





Bacteria were very numerous and were distributed both within and outside the sinusoids. The hepatic parenchymal cells of both the infected and control hens were markedly infiltrated with fat (Figures 9 and 11).

The pancreatic glands from the infected chickens appeared to be normal when examined grossly. Microscopically the only lesions were vascular congestion and intravascular bacteria.

Endocrine system

Gross examination of the thymus, thyroid and adrenal glands of the infected birds did not reveal lesions. Microscopic examination revealed moderate heterophilic infiltration of the adrenal and thyroid glands. Vascular congestion was observed in all three types of glands. Bacteria observed in the adrenal and thyroid glands were intravascular, but those in the thymus were both intravascular and extravascular. The islets of Langerhans of the pancreatic glands appeared to be normal.

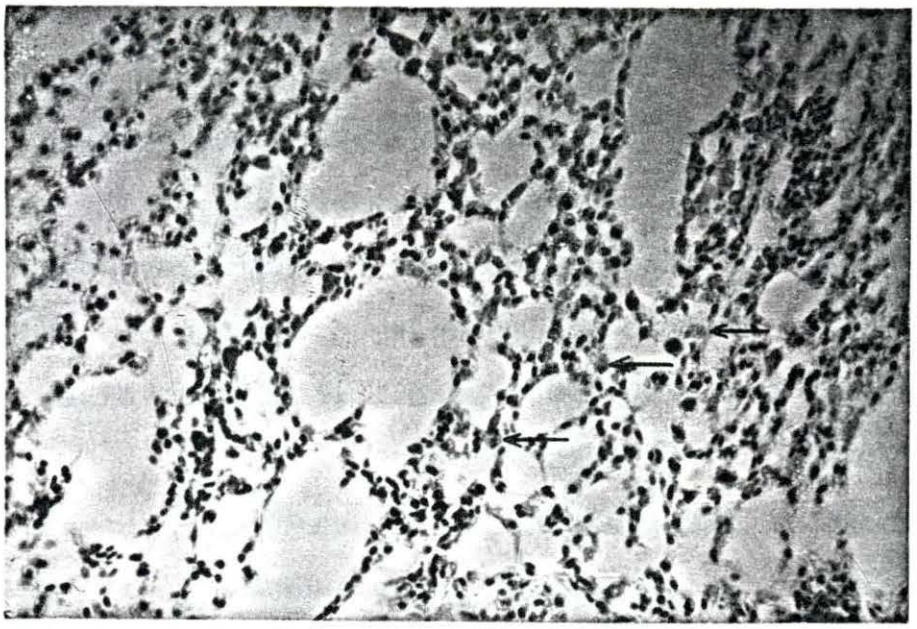
Respiratory system

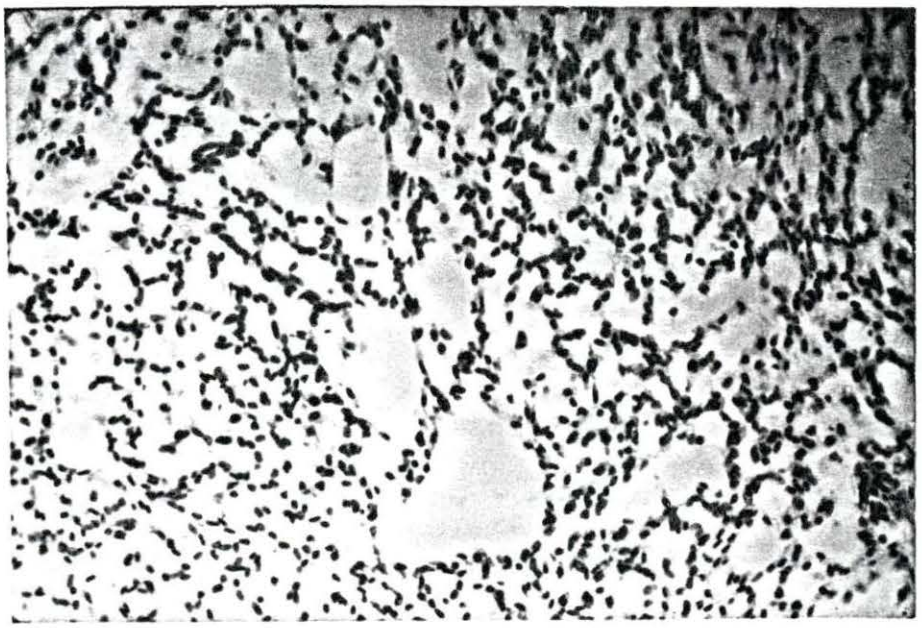
Gross examination of the pharynx and trachea of each of the infected chickens revealed increased amounts of mucus. No lesions were observed in the lungs and air sacs.

Microscopic examination of the lungs of the infected birds revealed moderate infiltration with heterophils (Figure 12).

Figure 12. Heterophilic infiltration in the lung of a rooster (no. 31) which died of acute fowl cholera. Arrows indicate the location of heterophils. Giemsa stain. x 280.

Figure 13. Lung from a control rooster (no. 35). Giemsa stain. x 280.





Few heterophils were found in the lungs of the control birds (Figure 13). There was no apparent difference in the amount of blood in the alveolar capillaries of the infected and non-infected birds. The veins of the infected birds were slightly dilated. Extravascular blood, indicative of hemorrhage, was observed in the tertiary and secondary bronchi of the lungs of both the control and infected chickens. Bacteria were quite numerous and were distributed throughout the lungs. Primarily they were found in the pulmonary capillaries and veins, but a few seemed to be in the alveolar spaces.

No pathologic changes other than hyperemia and intravascular bacteria were observed in the tracheas and air sacs of the infected birds. Focal areas of bacterial invasion of tissue with accompanying infiltration by heterophils were observed in the nasal turbinates of two of the infected males and one of the control males. No evidence of increased production of mucus was observed in the mucous glands of either the tracheas or nasal turbinates.

Uro-genital system

No lesions were observed grossly in the uro-genital system of the infected chickens. One of the control hens had a cystic remnant of the right oviduct.

Microscopic examination of the kidneys revealed vascular congestion and a very slight heterophilic infiltration. No degenerative or necrotic changes were observed. Bacteria were observed only within the blood vessels.

Vascular congestion was the only change observed in the testicles and epididymides of the males and the ovaries and oviducts of the females.

Skeletal system

No gross lesions were observed in the humeri and femurs. Microscopic lesions were limited to the marrow and were included in the description of lesions of the circulatory system.

Nervous system

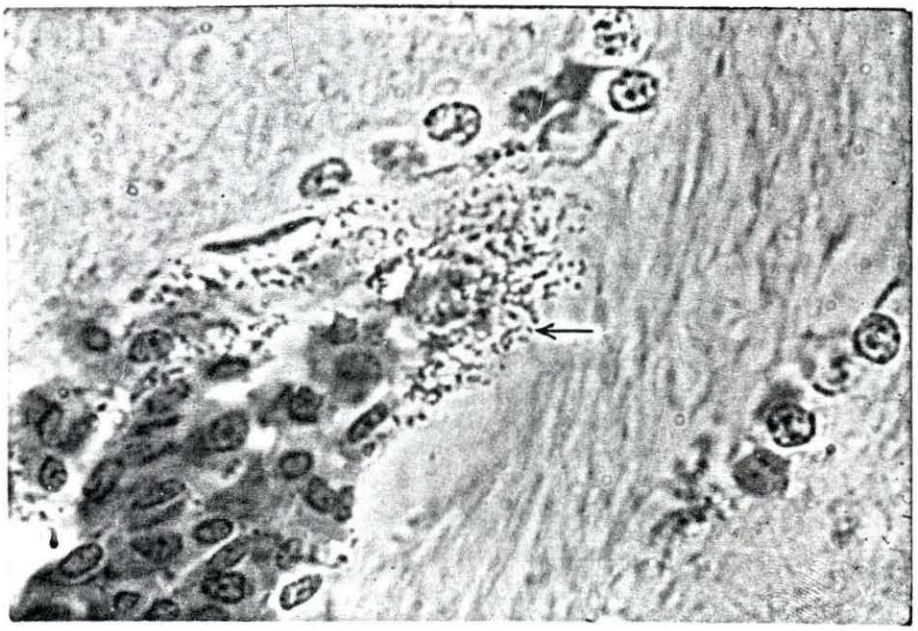
No gross lesions were observed in the nervous system. Sections of cerebrum, cerebellum, medulla and cervical, thoracic and lumbar areas of spinal cord were examined microscopically. The only lesions observed were vascular congestion and intravascular bacteria (Figure 14).

Integumentary system

Gross examination of the wattles revealed cyanosis. Examination of the conjunctiva revealed vascular congestion. No lesions were observed in the sternal bursas of the infected chickens.

Microscopic examination of the wattles, conjunctiva and sternal bursas of the infected birds revealed vascular congestion and intravascular bacteria.

Figure 14. Cerebral vessel of a hen (no. 52) which died of acute fowl cholera. Numerous intravascular bacteria are present. An arrow indicates the location of the bacteria. McCallum-Goodpasture stain. x 1120.



Muscular system

No gross lesions were observed in the cardiac, smooth or skeletal musculature. Microscopically there was congestion of veins, venules and capillaries with blood. Bacteria were present in the vessels.

DISCUSSION

The most pronounced and significant lesion observed in the infected chickens was generalized passive hyperemia. This term indicates a disturbance in circulation in which the capillaries and veins are dilated with blood (17). It represents a decreased venous outflow secondary to venous stasis (18).

According to Moon (19), if the dilation of capillaries and veins, by blood, is due to a damming back of the venous circulation the condition is termed passive congestion. If, however, the dilation is the result of atony and relaxation of the capillaries and venules the condition is called capillovenous hyperemia. The two conditions are identical in appearance of affected tissues. They differ in the mechanism by which they are produced.

Since the pulmonary involvement of the infected chickens appeared to be insufficient to impair circulation, the distention of veins and capillaries was the result of either cardiac insufficiency, loss of vascular tone, or both.

Shock has been defined by Robbins (18) as, "A common grave medical emergency characterized basically by a reduction in the effective circulating blood volume and in the blood pressure."

According to Sodeman (20), "true shock" is characterized by a reduced venous return and lowered blood pressure accom-

panied by a circulation impairment which tends to progress and eventuate in an irreversible circulatory failure and death.

The vascular dilatation observed in the infected chickens, irregardless of whether it resulted from cardiac insufficiency, atony of the veins and capillaries, or both, represents the changes which occur in shock.

The signs of disease observed in the chickens; depression, diarrhea, increased respiratory rate, cyanosis, coma, and death, are the same as the symptoms reported for shock in man (21).

The anatomic changes produced by shock in man involve chiefly the lungs, heart, liver, adrenals, and kidneys (18). Congestion and edema are often observed in the lungs, fatty degeneration in the myocardium and liver, lipid depletion in the adrenals, and degeneration and necrosis of the tubules of the kidneys. The earliest of these lesions to appear is not present until at least 18 hours after the onset of shock. With the exception of pulmonary venous congestion, the anatomic changes described for shock in man did not occur in the infected chickens. Because of the short time between the appearance of signs of disease and death of the infected chickens it is unlikely that there was time for the majority of the lesions described for man to occur.

Shock resulting from bacteremias in which Gram-negative

bacilli are involved is not uncommon (22, 23). This type of shock has been described as the result of the action of endotoxin released by thebacteria (21). The endotoxin is apparently a polymolecular phospholipid-polysaccharide-protein complex (24). A glyco-lipid substance, toxic for mice, was obtained from P. multicida by trichloroacetic acid extraction by Pirosky (25) in 1938.

The lesions described in this study were less severe and affected fewer tissues than those described for fowl cholera by other authors and referred to earlier in this paper. It is probable that these differences are related to differences in the acuteness of the disease. The chickens in this experiment died before some of the lesions previously described would have had time to develop.

The same principle may apply to the differences in degree of involvement between the males and females in this experiment.

The infected roosters survived an average of 7 hours after the onset of symptoms and developed more pronounced lesions than the hens which survived only an average of 3.5 hours after the onset of symptoms.

Coagulative necrosis, such as observed in the livers of the infected birds, is recognized histologically by persistence of the cell mass after the intracellular detail has disappeared. It may occur as a result of anoxia or toxins (18).

Either or both of these conditions could have been involved in the chickens; hypoxia as a result of impaired circulation, and toxins as a result of bacterial infection.

Heterophil infiltration, the avian counterpart to neutrophil infiltration in mammals, is a usual early response to tissue injury in birds. These cells are usually the most numerous type in leucocytosis resulting from bacterial infections in birds.

Hemorrhage into the bronchi of the control birds may have been the result of the severe muscular contractions which accompany death by electrocution. Similar hemorrhage in the infected birds may be related to the muscular contractions of agonal convulsions.

The focal areas of infection observed in the nasal turbinates of two of the exposed males suggested that these were sites of invasion. A similar lesion observed in one of the control males, however, discounts the importance of this observation. The reason for the extravascular bacteria in the thymus glands of the infected birds is not apparent.

Microscopic examination of the respiratory and digestive tracts, employing mucicarmine stained sections, did not reveal increased mucous gland activity even though an increased amount of material which resembled mucus had been observed grossly. Probably these glands were producing more mucus than normal, but since it was being secreted into the digestive and

respiratory tracts no increase in mucus was reflected within the glands.

The presence of more fat in the tissues of the hens than in the tissues of the roosters is a reflection of physical condition prior to the experiment and is not related to the P. multocida infection.

No lesions were observed in the central nervous system, and so no information was obtained on whether or not the central nervous system plays a part in controlling the pathogenesis of the disease as suggested by Movsesyan (11, 12) in his study of pasteurellosis in cattle.

Although heterophils had infiltrated the lungs of the infected chickens they did not appear to be present in sufficient number or location to block the pulmonary vessels as suggested by Stamatin et al., (13), in his report on pasteurellosis of sheep.

Since the most significant lesion observed was a vascular change indicated by dilatation of the venous system, and since shock produced by administration of Gram-negative bacilli endotoxin (26) also produces venous dilatation, it would seem that a study of the possibility of endotoxin production by P. multocida is indicated.

Shock resulting from bacterial infections is not necessarily a result of the direct action of endotoxins or exotoxins. Such things as products of host tissue damage may also be involved (24, 27).

SUMMARY

Twelve adult New Hampshire Red chickens were utilized in this experiment. Three roosters and 3 hens were exposed, by nasal cleft instillation, with Pasteurella multocida. The other 6 chickens, 3 roosters and 3 hens, were not exposed but were used as controls for evaluating changes in the exposed birds.

Signs of disease in the exposed birds in the order of appearnace were; depression, diarrhea, increased respiration rate, cyanosis, coma, and death.

The disease produced was acute, and resulted in death of all exposed birds 20 to 28 hours after exposure. The control birds were killed by electrocution, and necropsies were conducted on both the exposed and control birds.

Gross lesions in the infected birds were generalized passive hyperemia, multiple small areas of hepatic necrosis, increased amounts of mucus in the digestive and respiratory tracts, a few small subserous hemorrhages on the gizzard of one rooster, and marked congestion of the mid-portion of the small intestine of the males.

Thirty-eight sections representing 30 tissues were prepared from each bird and examined microscopically. Six stains; hematoxylin and eosin, Giemsa, Gomori's trichrome, mucicarmine, luxol-fast-blue periodic-acid-Schiff hematoxylin, and MacCallum

Goodpasture stain for bacteria, were used for evaluating tissue changes.

Microscopic lesions in the infected birds were generalized passive hyperemia, heterophilic infiltration of the lung, liver, adrenal, kidney, and thyroid, heterophilic depletion and hemopoietic cell degeneration in the bone marrow, generalized bacteremia, and acute focal necrotic hepatitis. The hyperemia was more pronounced in the males than in the females.

Many more heterophils were observed in the bone marrow of the normal roosters than were observed in the normal hens. Tissues of the hens contained more fat than did those of the roosters.

The most pronounced and significant of the lesions produced by the P. multocida infection was generalized passive hyperemia. Generalized passive hyperemia in this case resulted from cardiac insufficiency, atony of veins and capillaries, or both, and is indicative of the clinical syndrome of shock.

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